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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
15514 Pa		
International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/EP99/02463	13 April 1999 (13.04.99)	14 April 1998 (14.04.98)
International Patent Classification (IPC) or national classification and IPC G01N 33/68		
Applicant HASSAN, Jomaa		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22 October 1999 (22.10.99)	04 July 2000 (04.07.2000)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP99/02463

I. Basis of the report

1. This report has been drawn on the basis of (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

☒ the international application as originally filed.

☐ the description, pages 1-39, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____.

☐ the claims, Nos. 1-33, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. _____, filed with the letter of _____,
Nos. _____, filed with the letter of _____.

☐ the drawings, sheets/fig 1/20-20/20, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/fig _____

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP 99/02463

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

The sequence protocol (11 pages) is listed as pages 29-39 of the description.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: III

Claims 25-29 pertain to subject matter for which an international search report has not been established. Therefore, no international preliminary examination has been carried out in respect of these claims (PCT Rule 66.2(a)(vi)).

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-3,6,11,16-21,24,31-33	**YES**
	Claims	4,5,7-10,12-15,22,23,30	**NO**
Inventive step (IS)	Claims	1-3,6,11,16,17,20,21,24,31-33	**YES**
	Claims	4,5,7-10,12-15,18,19,22,23,30	**NO**
Industrial applicability (IA)	Claims	1-24, 30-33	**YES**
	Claims		**NO**

2. Citations and explanations

The following documents are referred to:

D1: Proc. Natl. Acad. Sci. USA. 95 (1998), 2100-2104, (Lange, B.M. *et al.*); XP002116672

D2: Proc. Natl. Acad. Sci. USA. 95 (1998), 2105-2110, (Lois, L.M. *et al.*); XP002116673

D3: Proc. Natl. Acad. Sci. USA. 94 (1997), 12857-12862 (Sprenger, G. *et al.*); XP002116674 (mentioned in the application)

D4: Tetrahedron Letters, Vol. 39 (1998), pages 23-26, (Putra, S.R. *et al.*); XP002116676

D5: Antibiotics and Chemotherapy 1997, pages 357-359, (Greenwood, D.); ISSN: 0570-3123; XP002113259

D6: Antimicrobial Agents and Chemotherapy, Vol. 22 (1982), pages 560-563, (Neu H.C. *et al.*); ISSN: 0066-4804; XP002113261

D7: Antimicrobial Agents and Chemotherapy, Vol. 19 (1981), pages 1013-1023, (Neu H.C. *et al.*); ISSN: 0066-4804; XP002113260

1. NOVELTY

Claims 4, 5, 7-10, 12-15, 22, 23 and 30 fail to meet the requirements of PCT Article 33(2) for the following reasons:

1.1 1-deoxy-D-xylulose 5-phosphate synthases, which are encoded by DNA sequences homologous with the protein-coding areas of the DNA sequence shown in Figures 1b and 2b and therefore hybridize with these, are disclosed by D1 (abstract; Figure 4), D2 (abstract; Figures 3 and 4), D3 (abstract; Figure 2) and D4 (abstract). The subject matter of **Claims 4 and 9** in the present application therefore lacks novelty within the meaning of PCT Article 33(2).

1.2 Owing to lack of clarity (cf. Box VIII, 4. and 5.), the subject matter of **Claim 5** is disclosed by all documents that disclose proteins.

1.3 Dependent **Claims 7 and 8** likewise lack novelty for the following reasons:

- 1-deoxy-D-xylulose 5-phosphate synthase produced by expression of an exogenous DNA, the DNA of which synthase hybridizes with the protein-coding areas of the DNA sequence shown in Figures 1b and 2b, is disclosed by D1 (abstract; page 2100, last paragraph-page 2102, line 5).

- Owing to lack of clarity (cf. Box VIII, 8.), the subject matter of Claim 8 is disclosed by all documents that disclose proteins.

1.4 Nucleic acids that code for 1-deoxy-D-xylulose 5-phosphate synthases and hybridize with the DNA sequence shown in Figures 1b and 2b are also disclosed by D1 (page 2100, penultimate paragraph), D2 (abstract; page 2107, right-hand column, lines 11-28; Figure 2), D3 (abstract; page 12859, right-hand column, lines 9-13) and D4 (abstract; page 26, Note 7). The subject matter of **Claim 10** therefore likewise lacks novelty within the meaning of PCT Article 33(2).

1.5 Recombinant expression vectors that contain the DNA of a 1-deoxy-D-xylulose 5-phosphate synthase and are expressed in *E. coli* are likewise disclosed by D1 (page 2100, last paragraph, line 2), D2 (page 2107, left-hand column, last four lines), D3 (page 12859, left-hand column, lines 5-11, and right-hand column, lines 9-21) and D4 (page 26, Note 7), as are host cells transfected with this DNA and the use of this DNA to transfect the host organism. **Claims 12-15** therefore also lack novelty.

1.6 A process for identifying a nucleic acid that codes for a 1-deoxy-D-xylulose 5-phosphate synthase, wherein a nucleic acid probe that hybridizes with the DNA sequence shown in Figures 1b and 2b is incubated with the probe

and hybridization is identified by means of a further reactant binding to the probe, is disclosed by D1 (page 2100, penultimate paragraph). The subject matter of **Claim 22** therefore likewise lacks novelty.

1.7 Dependent **Claim 23** likewise fails to meet the requirements of PCT Article 33(2), since pre-identification amplification of the nucleic acid to be identified is disclosed by D1 (page 2100, penultimate paragraph).

1.8 **Claim 30** also lacks novelty within the meaning of PCT Article 33(2), since the active substance 3-(N-formyl-N-hydroxyamino)propyl phosphonate (fosmidomycin or FR-31564) is disclosed by D5 (page 359, right-hand column), D6 (abstract) and D7 (abstract).

2. INVENTIVE STEP

2.1 **Claims 18 and 19** do not meet the requirements of PCT Article 33(3), since they pertain to antibodies to proteins from the DXP metabolic pathway and to their production. However, the production of antibodies to a known protein (see 1.1 above) is a conventional process which a person skilled in the art is accustomed to implementing on the basis of routine considerations, especially as the advantages conferred are readily foreseeable. Consequently, the subject matter of these claims does not involve an inventive step.

2.2 Conversely, **Claims 1-3, 6, 11, 16, 17, 20, 21 and 24** involve an inventive step within the meaning of PCT Article 33(3) for the following reasons:

The process described in **Claim 1** differs from the subject matter of D1, which represents the closest prior art, in that it enables the selection of antiparasitic agents. The technical problem addressed by the invention may be seen to consist in identifying antiparasitic agents. Although D1 mentions the possibility of identifying highly specific antibiotics and herbicides using the DXP metabolic pathway (last paragraph, sentence 2), neither D1 nor any of the other citations suggest that parasites possess a DXP metabolic pathway. Therefore, a person skilled in the art would neither be motivated nor guided technically to use this metabolic pathway in investigating possible new antiparasitics. Therefore, the solution proposed in Claim 1 may be considered to involve an inventive step (PCT Article 33(3)).

For analogous reasons **Claim 6** also appears to involve an inventive step, since purification of DXP metabolic proteins from parasites is not suggested by the relevant citations. **Claim 11** may also be considered to involve an inventive step, since the relevant citations give no suggestion of a DXP metabolic pathway in parasites. Likewise **Claims 20, 21 and 24** appear to involve an inventive step, since

they pertain to the use of proteins, antibodies or test systems for identifying antiparasitic agents.

Claims 2, 3, 16 and 17 are dependent on Claim 1 and therefore likewise meet the requirements of the PCT with respect to inventive step.

2.3 **Claims 31-33** also appear to involve an inventive step (PCT Article 33(3)) provided that they refer to Claim 30. The reasons for this are as follows:

Claim 31 pertains to the use of an antiparasitic agent identified as per the invention to produce a drug for treating parasitic infections ("second medical use"). Since the relevant citations give no indication of a DXP metabolic pathway in parasites, this use is not obvious and it consequently involves an inventive step.

Claims 32 and 33 are dependent on Claim 31 and therefore likewise meet the requirements of the PCT with respect to inventive step.

3. INDUSTRIAL APPLICABILITY

The subject matter of Claims 1-24 and 30-33 appears to be industrially applicable and therefore meets the requirements of PCT Article 33(4).

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: VI

Filing no. Patent no.	Publication no. (day/month/year)	Filing date (day/month/year)	Priority date (validly claimed) (day/month/year)
DE-A-197 52 700	02.06.1999	28.11.1997	N/A
DE-U-298 00 547	20.05.1999	16.01.1998	28.11.1997

The indicated patent and the indicated utility model were published after the filing date of the present application, but have a filing or priority date preceding the priority date of the present application. Therefore, these documents could become relevant in the national or regional phase of the present application.

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Pursuant to PCT Rule 5.1(a)(ii), the description should cite the documents D1, D2 and D4-D7 and briefly outline the relevant prior art contained therein.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1. The expression "or equally acting derivatives thereof" used in **Claim 1** is vague and unclear and leaves the reader in doubt as to the chemical features of these derivatives. Consequently, the definition of the subject matter of these claims is unclear (PCT Article 6).

2. The subject matter of **Claim 2** does not meet the requirements of PCT Article 6, since the proteins involved in the indicated steps are not fully supported by the description.

3. Although **Claim 3** is drafted as a dependent claim, the subject matter of this claim is not contained in the preceding claims, since inhibition of an enzyme (see Claim 1) includes neither inhibition of the production of this enzyme nor promotion of its degradation (Claim 3). For these reasons Claims 3 does not meet the requirements of PCT Article 6.

4. Further, the expressions "of the participant enzymes" and "of the participant cofactors" in **Claim 3** are not clear, since it is not clear what these enzymes and cofactors are participating in.

5. The expression "or fragments of these DNA sequences" in **Claims 4, 5 and 7** is unclear

VIII. Certain observations on the international application

and does not meet the requirements of PCT Article 6 because, if a minimum fragment size is not indicated, the matter for which protection is sought is not clearly defined. Since an individual base should also be considered a "fragment of these DNA sequences", the subject matter of Claims 4, 5 and 7 is anticipated by all known proteins.

6. A similar objection concerning lack of clarity refers to the expression "[...] sequences that hybridize with the [...] DNA sequences shown" in **Claims 4, 5, 7, 10 and 22**. The matter for which protection is sought by these claims is unclear unless the hybridization conditions are indicated, since all DNA sequences hybridize with each other under appropriately nonrestringent conditions.

7. The subject matter of **Claim 6** does not meet the requirements of PCT Article 6, since the description does not support "further proteins involved in the DXP metabolic pathway". Moreover, Claim 6 is unclear (PCT Article 6), since the matter for which protection is sought is not clearly defined for the following reasons. The wording "proteins involved in the DXP metabolic pathway" does not enable a person skilled in the art clearly to establish which proteins fulfil this function and which do not.

VIII. Certain observations on the international application

8. **Claims 7 and 10** likewise fail to meet the requirements of PCT Article 6, since the meaning of the expression "[...] sequences that hybridize with [...] without degeneration of the genetic code" is unclear. Since the hybridization of two DNA sequences is independent of the genetic code of the genes they encode, the sequences to which these claims pertain are not evident to a person skilled in the art. The matter for which protection is sought in these claims is therefore not sufficiently clearly defined.

9. The matter for which protection is sought in **Claim 8** is also unclear, since all proteins consist "of amino acids of the sequences [...]", that is, of the twenty protein-forming amino acids.

10. **Claim 12** is unclear within the meaning of PCT Article 6, since the wording "recombinant expression vector that contains DNA, which codes for [...] and [...] expresses the protein-coded DNA in an animal or a plant" is ambiguous, since it is not clear whether the vector or the DNA expresses the protein-coded DNA.

11. **Claim 19** is unclear, since the antibody is defined in relation to immunization "with a protein as per one of the preceding claims", although Claim 11 does not mention a protein. A similar objection applies to **Claim 24**,

VIII. Certain observations on the international application

since Claims 11 and 21 do not mention a protein.

12. The dependence of **Claim 23** is unclear, since this claim refers back to itself ("process according to Claim 23").

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TITLE: Identification of a thiamin-dependent synthase in Escherichia coli required for the formation of the 1-deoxy-D-xylulose 5-phosphate precursor to isoprenoids, thiamin, and pyridoxol.

AUTHORS: Sprenger GA; Schorken U; Wiegert T; Grolle S; de Graaf AA; Taylor SV; Begley TP; Bringer-Meyer S; Sahm H

AUTHOR AFFILIATION: Institut fur Biotechnologie 1 des Forschungszentrums Julich, Germany. sprenger@ibt.fz-juelich.de

SOURCE: Proc Natl Acad Sci U S A 1997 Nov 25;94(24):12857-62

CITATION IDS: PMID: 9371765 UI: 98058734

ABSTRACT: In Escherichia coli, 1-deoxy-D-xylulose (or its 5-phosphate, DXP) is the biosynthetic precursor to isopentenyl diphosphate [Broers, S. T. J. (1994) Dissertation (Eidgenossische Technische Hochschule, Zurich)], thiamin, and pyridoxol [Himmeldirk, K., Kennedy, I. A., Hill, R. E., Sayer, B. G. & Spenser, I. D. (1996) Chem. Commun. 1187-1188]. Here we show that an open reading frame at 9 min on the chromosomal map of E. coli encodes an enzyme (deoxyxylulose-5-phosphate synthase, DXP synthase) that catalyzes a thiamin diphosphate-dependent acyloin condensation reaction between C atoms 2 and 3 of pyruvate and glyceraldehyde 3-phosphate to yield DXP. We have cloned and overexpressed the gene (dxs), and the enzyme was purified 17-fold to a specific activity of 0.85 unit/mg of protein. The reaction catalyzed by DXP synthase yielded exclusively DXP, which was characterized by 1H and 31P NMR spectroscopy. Although DXP synthase of E. coli shows sequence similarity to both transketolases and the E1 subunit of pyruvate dehydrogenase, it is a member of a distinct protein family, and putative DXP synthase sequences appear to be widespread in bacteria and plant chloroplasts.

MAIN MESH HEADINGS:
Escherichia coli/*enzymology
Pentosephosphates/*metabolism
Pyridoxine/*biosynthesis
Terpenes/*metabolism
Thiamine/*biosynthesis

	Transferases/*metabolism
ADDITIONAL MESH HEADINGS:	**Amino Acid Sequence** **Arabidopsis/enzymology** **Escherichia coli/genetics** **Human** **Molecular Sequence Data** **Recombinant Proteins/genetics** **Recombinant Proteins/metabolism** **Sequence Homology, Amino Acid** **Support, Non-U.S. Gov't** **Support, U.S. Gov't, P.H.S.** **Transferases/genetics** **1997/12** **1997/16 02:41**
PUBLICATION TYPES:	**JOURNAL ARTICLE**
CAS REGISTRY NUMBERS:	**EC 2. (Transferases)** **EC 2.2.1.- (deoxyxylulose-5-phosphate synthase)** **0 (Pentosephosphates)** **0 (Recombinant Proteins)** **0 (Terpenes)** **0 (1-deoxyxylulose 5-phosphate)** **59-43-8 (Thiamine)** **65-23-6 (Pyridoxine)**
LANGUAGES:	**Eng**
GRANT/CONTRACT ID:	**DK44083/DK/NIDDK**